FSTC-HT-23-603-68

U.S. ARMY FOREIGN SCIENCE AND TECHNOLOGY CENTER

MASS BREEDING OF AEDES AEGYPTI MOSQUITOES

COUNTRY: USSR

TECHNICAL TRANSLATION

1C

TECHNICAL TRANSLATION

FSTC-HT-23- 603-68

MASS BREEDING OF AEDES AEGYPTI MOSQUITOES

by

V. V. Vladimirova

SOURCE: MEDITSINSKAYA PARAZITOLOGYA I PARAZITARNYYE BOLEZNI
(Medical Parasitology and Parasitic Diseases)
Vol. 35, No. 6, pp. 719-723, 1966
USSR

Translated for FSTC by ACSI

UNCLASSIFIED
Security Classification

AD 687757

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Foreign Science and Technology Center
US Army Materiel Command
Department of the Army

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
MASS BREEDING OF AEDES AEGYPTI MOSQUITOES

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Translation

5. AUTHOR(S) (First name, middle initial, last name)
V. V. Vladimirova

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
2 May 69	6	N/A

8a. CONTRACT OR GRANT NO.

b. PROJECT NO.

c. 9223628 2301

d. SMUFD-PR-I

9a. ORIGINATOR'S REPORT NUMBER(S)
FSTC-HT-23-603-68

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
ACSI Control Number J-6309

10. DISTRIBUTION STATEMENT
This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
US Army Foreign Science and Technology Center

13. ABSTRACT

A method for mass breeding of Aedes Aegypti is described. The results were obtained with larvae grown with an infusion of guinea pig excrement with brewer's yeast, in which a rich microflora developed. Specially constructed closets used for breeding mosquitoes and containers for growing larvae are described. This method permits one to obtain thousands of mosquitoes of known age and physiological condition per week, with little effort, time, or space. This method may also be used for breeding certain species of Anopheles and Culex.

DD FORM 1473

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Mass Breeding Mosquitoes Aedes Aegypti Insecticide Testing						

MASS BREEDING OF AEDES AEGYPTI MOSQUITOES

Of all the mosquitoes being propagated under laboratory conditions, the most information has been obtained with Aedes aegypti L. thanks to its fertility and simplicity. The larvae of the mosquito are successfully developed in saprobic media. The adult mosquitoes mate in small breeding sites and lay the eggs over the course of the entire year. The eggs retain their viability in the dry form for a long time. This makes it possible to store the egg masses and when needed to obtain a large number of winged insects simultaneously.

Much material has been published, devoted to culture of this species of mosquito. According to the data of the majority of authors, in order to obtain egg masses the mosquitoes are fed the blood of small animals, (rabbits, guinea pigs, hamsters), immobilized at the breeding sites with the mosquitoes or anesthetized with nembutal (Kirkwood, 1961; Porter at al, 1961). Filter paper is often used as the substrate for laying the eggs, by inserting it into a vessel with water, in a petri dish on moistened cotton, and less frequently - on small wood planks (Demina et al, 1951; Fay, 1964). The water was tinted with edible dyes so that the mosquitoes would not lay their eggs there (Williams and De Long, 1961).

The eggs, placed on the filter paper or other substrate, are usually kept on the moist medium for two days to ripen, and then dried. The egg masses are stored at room temperature and humidity or in closed glass vessels at a humidity of 80-90% (Burgess, 1959). The per cent of larvae which hatch by the second method is significantly higher (Judson, 1960). However, it should be noted that in a series of cases under these conditions mold appears, which is undesirable.

The eggs retain their viability for several months, but the per cent of larvae which hatch from them decreases as the storage time increases.

For simultaneous and 100% hatching of the larvae a series of authors recommend use of boiled refrigerated water, since it has been established that even a negligible reduction in oxygen concentration in the water stimulates the release of larvae from the eggs. Therefore, in several laboratories the egg masses are placed in narrow-necked vessels with distilled freshly boiled water (Judson, 1960) or in water to which ascorbic acid is added. Fresh infusions from leaves and grass give good results. It has been shown that the presence of amino acids and proteins in these solutions stimulate the hatching of the larvae (Borg and Horsfall, 1953; Weissman-Strum, 1962; Gjullin et al, 1939).

Abroad, the most frequently used food for the larvae is powder from biscuits (for dogs) or infusions from hay, bread or sugar with the addition of dry brewer's yeast or mouth excrement (Morlan et al, 1963; Kirkwood, 1961; Muspratt, 1962). Donets et al (1965) proposed a mixture of dry milk, brewer's yeast and bee bread with added penicillin as a standard medium for Ae. aegypti larvae. Akov (1962) used synthetic medium which contained solid casein, cholesterol, ribonucleic acid, inorganic salts and vitamins. But the use of this complicated medium is practically unjustified.

In order to obtain a population of mosquitoes of the same age, pupas are selected from the flasks. In order to facilitate this tedious work several methods have been proposed. A mechanical separator is used, for example, with a regulated opening through which larvae pass and pupas are retained (Fay and Morlan, 1959), or Ferric Oxide is added to the vessels with the larvae, and is swallowed by the larvae and they are separated with the help of a magnet. One to one and one-half hours before pupation the Ferric Oxide is taken out of the body of the larvae (Bar-Zeev and Galun, 1961; Fay et al, 1963). Insertion of the larvae into cold water (3-4°) gives good results with certain species of mosquitoes. The larvae immediately sink to the bottom, and the pupas remain on the surface. Cooling does not have a harmful effect on the further development of the mosquitoes. By this method one can separate pupas of certain species of Anopheles (A. stephensi; A. subpictus; A. quadrimaculatus; A. sinensis) and Culex (C. fatigans; C. pipiens pipiens; C. pipiens pallens; C. tarsalis; C. tritaeniorhynchus). But it is not very effective with Ae. aegypti (Ramakrishnan et al, 1963; Weathersby, 1964).

Adult mosquitoes are kept in breeding sites of various sizes. They are given sugar solution, honey or moistened raisins as the carbohydrate food (Porter et al, 1961).

In order to conduct experimental research, in particular in order to test new repellents and insecticides, it is necessary to have a large number of mosquitoes at one's disposal. With this purpose we have developed a method of mass breeding the Ae. aegypti mosquitoes. We have successfully maintained a culture of these mosquitoes since 1962. The eggs are obtained from the London School of Hygiene and Tropical Medicine, where mosquitoes were brought in 1926 from West Africa.

At the suggestion of P. G. Sergiev and A. A. Potapov, we began to conduct cultivation in walled cupboards with the use of special tanks for growing the larvae (by the modified method of McKiel, 1957). The tanks were made from galvanized iron with a capacity of 50 l. (Figure 1). The dimensions can be varied, but for an increase in fodder capacity and better aeration it is expedient to make them wider. Filling of the tanks depends on the working intensity of the culture, i.e. on the number of developing larvae. There are three narrow apertures on the roof. In one of these there is a rubber stopper, through which the infusions are poured and the fresh batch of larvae is added (a large funnel from the right side). Through the second passes a rubber stopper for supplying air and a lamp cord. The third serves for holding the thermometer and addition of dry fodder.

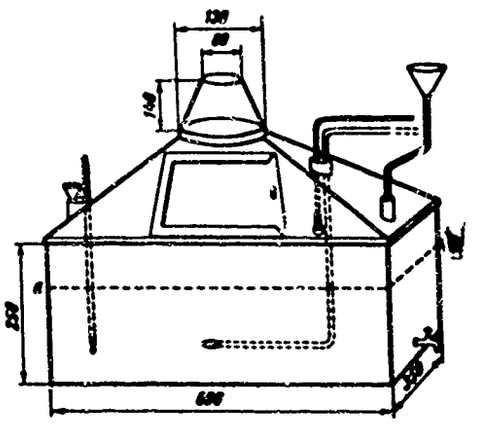

Figure 1
Tank for Growing Larvae
a, b.- Level of nutritive infusion.

The nutritive infusion for loading of the tank is prepared by the method of the London School of Hygiene and Tropical Medicine. For this, 15-16 g of powder from dried guinea pig excrement ground in a coffee mill and one teaspoon of fresh brewer's yeast mixed in one l of tap water kept for one day at room temperature in order to remove chlorine. After five days 0.5 l of water is poured into the feed. The feed is aged in wide vessels at room temperature for seven days after addition of the water. Infusoria and bacteria are liberally generated in it, which serves as the basic food for the larvae. In addition the larvae are fed on ground (to dust) powder from dry water fleas or gamma-sumac. The contents of the tank was changed each week. For this purpose, one-third of the infusion was poured off through a tap and fresh added.

After two to three months the tank was washed and refilled. For observation through the infusion there is a door or organic glass in the cover, covered over with black paper. Inflow of air for aeration of the fluid and for prevention of formation of a film, occurs by microcompressors which with the help of a watch mechanism is engaged for 10-20 minutes per hour.

Control after control is carried out by means of periodic sampling of the fluid from the tank (once a week). The pH and the presence of microorganisms is determined on a sample. As it is used the pH of the medium shifts to the alkaline side (pH from 7.0-7.2 to 7.5-8.0). Moreover, the color of the fluid changes from greenish-brown to dark brown. A change in these indices, and also the disappearance of microorganisms in the infusion indicate the necessity for changing the nutritive medium. For continuous escape of mosquitoes in the tank it is necessary to maintain uniform ratio of larvae of young and old ages. With insufficient food the larvae become small and insufficient viable mosquitoes.

The walled closets (Figure 2) are made out of plywood, with tightly closing doors and loading platforms of white plastic. The doors and rear wall are glassed. The lower portion of the closet is used for breeding purposes. On the first platform are 1-2 tanks, on the remaining sections are located breeding tanks, (60 x 40 x 40 cm) of various purposes: a) receptacles (over the tank), b) experimental, c) mothers with crystallizing basins for laying the eggs.

The temperature in the closets is maintained at 26-28° (with electrolamps) and the relative humidity is 70-80%

(by cuvettes with water). For more uniform distribution of heated and humidified air one of the platforms divides the closets into two isolated halves (an upper and a lower). On the outside the glassed doors of the closets are covered with black paper.

Cultivation of mosquitoes begins with breeding of the larvae in crystallizing basins with water, which were placed on the platforms in the closets. When the larvae reached the second growth, they were filtered through fine gauze and transferred to tanks with infusion. The development of the eggs to imago usually lasts 8-9 days. The released mosquitoes pass through the cone in the cover of the tank into the breeding sites. In order to stimulate flight the cone is illuminated from the side by a lamp. Therefore, this method eliminates the operation of transplanting the pupas. For work in testing repellents in the laboratory we obtain 2000 to 3000 mosquitoes every 3-6 days. But the number of mosquitoes hatching may be higher as a result of an increase in the density of larvae in the tank. The ratio of males and females in the first three days of hatching is approximately 1:1, after two weeks the males lead by 20-30%, and after one month - 1-10%, the females live 1½-2 months. In the breeding sites with the mosquitoes designated for testing of repellents, tanks with cotton, moistened with 10% glucose solution are placed. Mosquitoes selected for laying eggs, beside the glucose, are daily allowed to feed on blood (guinea pig or white mouse). For this purpose, guinea pigs anesthetized with nembutal are placed in the tanks for 3-4 hours, mice are placed inside the breeding tanks in cylinders with metal screening.

For laying eggs, crystallizing basins with water tinted with nutritive infusion, are placed in the maternal tanks. Small wood planks (7 x5 cm) 0.5 cm thick, suspended in rows on wire rods, placed on the crystallizing basins (one end of the planks immersed in the water) or jugs, cut from large cork stoppers, serve as the substrate for the laying of eggs. After 2-3 days the planks with the eggs on them are removed from the water and remain for two days in the closet at a humidity of 70-80% for aging of the eggs, and then are dried and kept under room conditions. In this form the eggs retain their viability for at least six months. As required, planks or corks are placed in the crystallizing basins with water with the egg masses, and the culture is renewed.

Literature

Demina, M. A., P. B. Levitanskaya, Va Avramenko, Med. Paravitol. No. 5, p. 472, 1951.
Donets, V. S., N. G. Dashkina, Vm Loskot et al, Ibid. No. 5, p. 518, 1965.
Akov, S. Journ. Insect. Physiol., Vol. 8, p. 319, 1962.
Bar-Zeev, M., Galun, R., Mosquito News, Vol. 21, p. 225, 1961.
Borg, A., Horsfall, W., Ann. Entomol. Soc. Amer., Vol. 46, p. 472, 1953.
Burgess, L. Mosquito News, Vol. 19, p. 256, 1959.
Fay, R., Ibid., Vol. 24, p. 300, 1964.
Fay, R., Morlan, H. B., Ibid., Vol. 19, p. 144, 1959.
Fay, R., McCray, E., Kilpatric, J., Ibid., Vol. 23, p. 210, 1963.
Gjullin, C. Yates, W., Stage, H., Science, Vol. 89, p. 539, 1939.
Judson, C., Ann. Entomol. Soc. Amer., Vol. 53, p. 688, 1960.
Kirkwood, A., Lab. Pract., Vol. 10, p. 289, 1961.
McKiel, L. Mosquito News, Vol.17, p. 255, 1957.
Morlan, H. B., Hayes, R. O., School H.F., Publ. Hith Rept. (Wash), Vol. 78, p. 711, 1963.
Muspratt, J. J., Entomol. Soc. S. Afr., Vol. 25, p. 82, 1962.
Porter, J. Korsuchi, G., Kuck, M., Mosquito News, Vol. 21, p. 350, 1961.
Ramakrishnan, S., Krishnamurthy, B., Singh, N., Indian J., Malar., Vol. 17, p. 119, 1963.
Weathersby, A., Mosquito News, Vol. 23, p. 242, 1964.
Weissman-Strum, A., Kindler, S., Nature, Vol. 196, p. 231, 1962.
Williams, R. E., DeLong, D. M. J. econ. Entomol., Vol. 54, p. 2265, 1961.

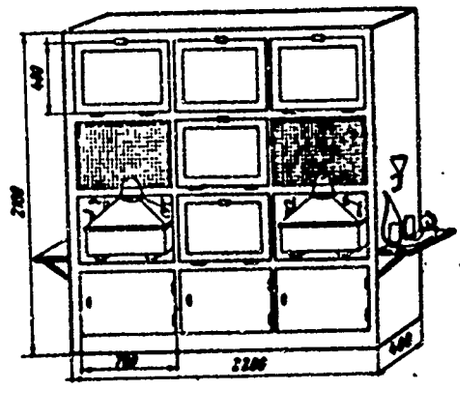

Figure 2

General view of Mosquito Cupboard